

Metabolome profiling of *Lagenaria siceraria* leaves extracts of different growth regulators using UPLC/MS/MS analysis



PG-09

Nour S. Al-Shaikh, Hala H. Zaatout*, Shaimaa M. Sallam,
Amr M. El-Hawiet and Hala M. Hammoda

Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt

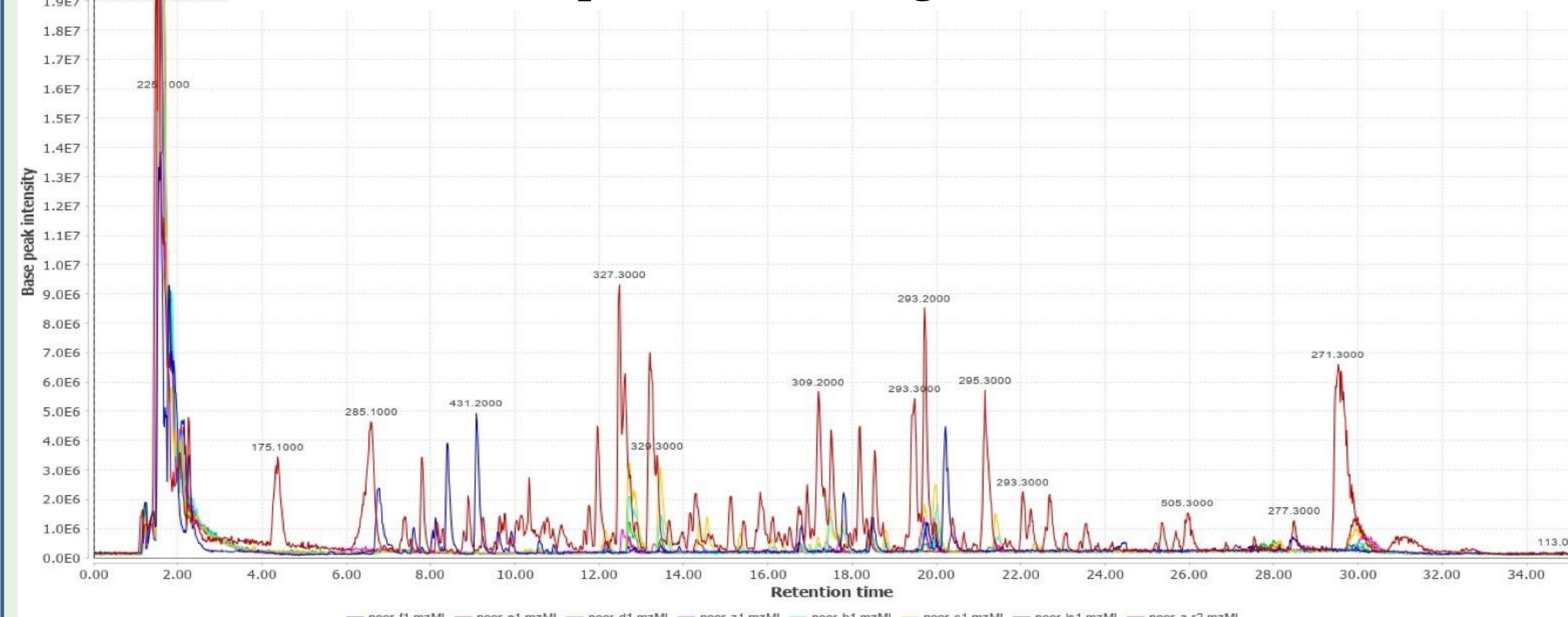
Introduction

Lagenaria siceraria (Mol.) Standl, Family Cucurbitaceae, commonly known as bottle gourd, is a medicinal plant widely cultivated in African and Asian countries. There is a great interest in its pharmacological activities and the corresponding bioactive constituents which has an amazing and significant ability for the treatment of various diseases. Plant tissue culture represent a potential renewable source of valuable medicinal compounds. This study was conducted to investigate organogenesis from *L. siceraria* explants and the effects of various plant growth regulators on its metabolic profile.

Materials and Methods

The present work involves the application of a powerful LC-ESI-MS/MS separation system coupled with a TQD mass analyzer for the comprehensive characterization of leaves extracts of Palestinian *L. siceraria*. Various plant growth regulators were used; 2,4-D, NAA, IAA, GA, 2,4-D+NAA, 2,4-D+IAA, 2,4-D+GA. Assignment of secondary metabolites of *L. siceraria* leaves and their corresponding hormonal cultured extracts were performed using an UPLC XEVO TQD triple quadrupole instrument Waters Corporation, Milford, MA01757 U.S.A. a triple quadrupole (TQD) mass spectrometer in conjunction with electrospray ionization (ESI) source were exploited to analyze the samples in a negative ionization (NI) mode. The negative ion mode was chosen as it revealed a higher sensitivity, more observable peaks, so that, wide range of structural information can be obtained. Metabolite assignments were determined according to their corresponding MS data (quasi-molecular ions as well as diagnostic MS/MS fragmentation pattern compared with our in-house database, reference literature, phytochemical dictionary of natural products database (DNP).

UPLC-MS metabolite profiling data from all *L. siceraria* samples the detected compounds according to their retention times.



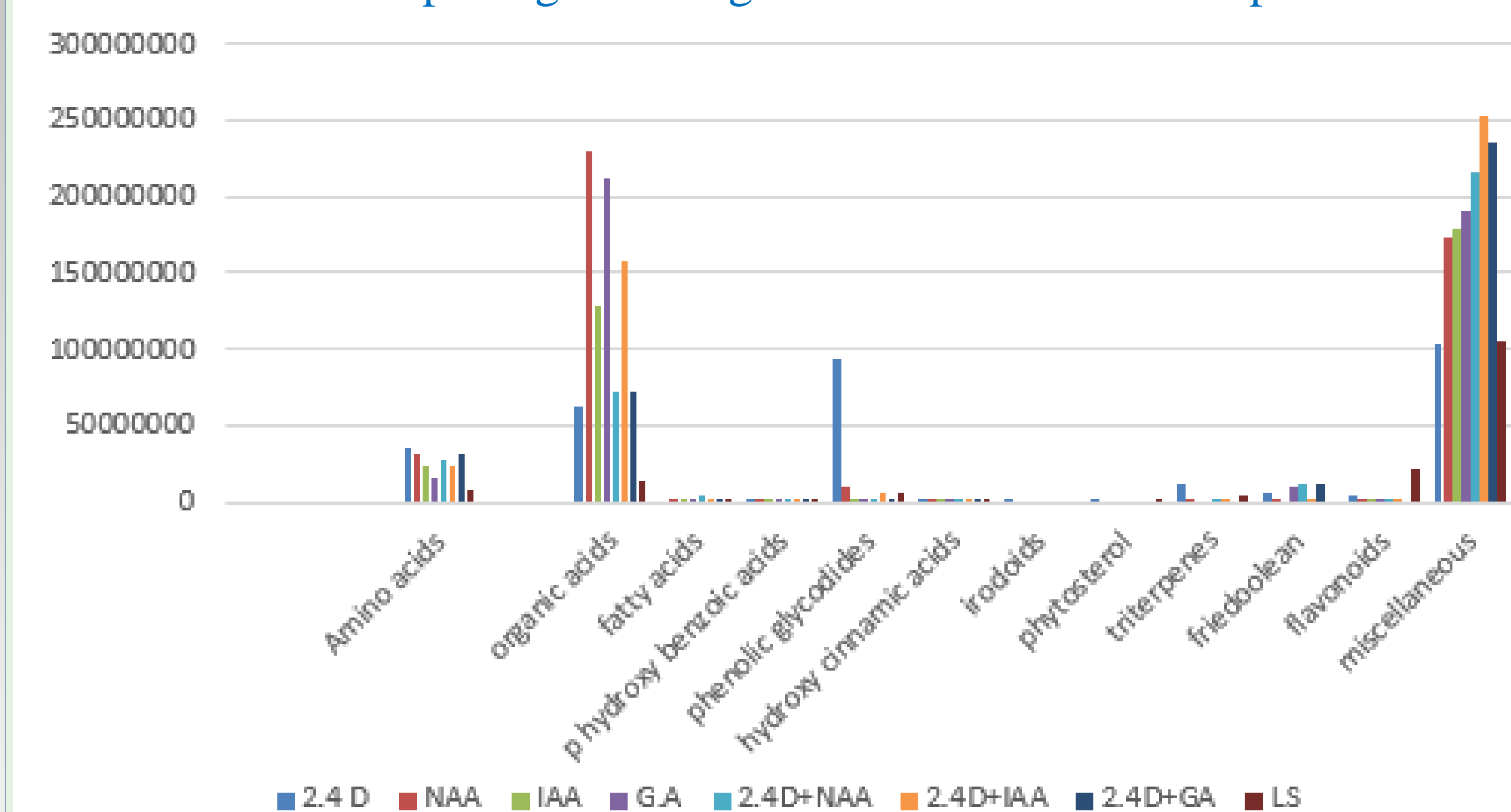
Overlay of total ion chromatograms (TIC) for chromatographic separation of different callus extracts of *L. siceraria* distinguished by different line pattern.

L. siceraria, 2,4-D, NAA, IAA, GA, 2,4-D+NAA, 2,4-D+IAA, 2,4-D+GA.

Results

Seventy four metabolites were annotated in bottle gourd via UPLC/MS/MS analysis. These Metabolites were belonging to terpenoids, flavonoids, amino acids, organic acids, and phytosterols classes.

LC-MS/MS comparison between different extracts of *L. siceraria* exhibited the effect of plant growth regulators on the metabolic profile.



- It was observed that Amino acids were appeared extensively, in different plant growth regulators samples
- Mass analysis proved that phenolic glycosides were the highest in 2,4-D sample.
- On the other hand, triterpenes were found in 2,4-D followed by NAA samples, while, mixture of 2,4-D+GA, 2,4-D+NAA and GA samples showed highest amount of friedoleane. Flavonoids were better detected in the methanolic extracts of *L. siceraria* leaves followed by 2,4-D sample
- The most remarkable observation was the higher concentration of organic acids in all growth regulators samples especially, NAA followed by GA then combination of 2,4-D+IAA
- It was found that plant growth regulators enhanced the production of different metabolites compared to the ethanolic extract of *L. siceraria* leaves and 2,4-D sample was the richest extract. *Lagenaria* leaves extracts of different growth regulators exhibited significant antioxidant activities using DPPH technique.

Conclusions

This study concerning tissue culture and metabolomic profiling of the resulted callus of *Lagenaria siceraria*. It was found that plant growth regulators enhanced the production of different metabolites compared to the ethanolic extract of *L. siceraria* leaves and 2,4-D sample was the richest extract. It is worth mentioning that tissue culture study together with metabolomic profiling is done for the first time in genus *Lagenaria*.

Our established method of analysis displayed a considerable improvement in analytical effectiveness, sensitivity and time where most of compounds were efficiently separated in less than 32 min This provides new possible attributes for in nutrition and health-care fields

References

1. Minocha, S., An overview on *Lagenaria siceraria* (bottle gourd). *Journal of Biomedical and Pharmaceutical Research*, (2015). 4(3): 4 -10
2. Hala Zaatout, Nour ALShaikh, Shaimaa Sallam, Hala Hammoda, Phytochemical And Biological Activities of *Lagenaria siceraria*: An Overview, *Egypt. J. Chem.* (2023), 66(10), 479 - 495
3. Jaiswal, R. and N. Kuhnert, Identification and characterization of the phenolic glycosides of *Lagenaria siceraria* Stand.(bottle gourd) fruit by liquid chromatography –tandem mass spectrometry. *Journal of agricultural and food chemistry*, (2014), 62(6): 1261 -1271.
4. Sakna, S. T., A. Mocan, H. N. Sultani, N. M. El-fiky, L. A. Wessjohann and M. A. Farag "Metabolites profiling of Ziziphus leaf taxa via UHPLC/PDA/ESI-MS in relation to their biological activities." *Food chemistry* (2019), 293: 233-246
5. Bankova, V., D. Bertelli, R. Borba, B. J. Conti, I. B. da Silva Cunha, C. Danert, M. N. Eberlin, S. I Falcão, M. I. Isla and M. I. N. Moreno "Standard methods for Apis mellifera propolis research." *Journal of Apicultural Research* (2019). 58(2): 1-49.
6. Gonda, I., E. Bar, V. Portnoy, S. Lev, J. Burger, A. A. Schaffer, Y. a. Tadmor, S. Gepstein, J. J. Giovannoni and N. Katzir "Branched-chain and aromatic amino acid catabolism into aroma volatiles in Cucumis melo L. fruit." *Journal of experimental botany* (2010), 61(4): 1111-1123.